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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: CHATFIELD
Serial No. 09/646,925
Filed: January 31, 2001
For: BACTERIA ATTENUATED BY A NON-REVERTING MUTATION IN EACH OF THE AROC, OMPF AND OMPC GENES, USEFUL AS VACCINES

DECLARATION

The Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

I, Michael Darsley, declare as follows:

1. I currently hold the position of Vice President of Bacterial Research at Acambis plc, the assignee. I joined Acambis in 1996 after seven years with Igen Inc., a US biotechnology company. I have a BA in Biochemistry and a DPhil in Molecular Immunology from Oxford University. My area of interest is the development of prophylactic and therapeutic vaccines for infectious diseases and allergies, with a particular focus on live, attenuated bacterial vaccines.

2. I have reviewed the patent application in question and the "Official Action" dated 12th December 2002. The patent application is concerned with bacteria, particularly *E.coli* bacteria, attenuated by a non-reverting mutation in each of the *aroC* gene, the *ompF* gene and the *ompC* gene. The bacteria are useful in vaccines.

3. I attach as Exhibits 1 and 2 reports of two clinical trials carried out on a

vaccine as described in the patent application. (The table of contents and some of the appendices are missing from the report of the first trial (Exhibit 1), but the key information is present.)

4. In both trials, two strains of bacteria were tested, strain "PTL-ETEC-002" and strain "PTL-ETEC-003". Strain PTL-ETEC-002 is attenuated by mutations in the *ompR* and *aroC* genes, and is outside the scope of the invention claimed in the patent application. PTL-ETEC-003 is attenuated by mutations in *aroC*, *ompC* and *ompF* and is within the scope of the invention.

5. The first trial showed that both the *aroC ompR* and *aroC ompC ompF* mutant strains were safe and immunogenic.

6. The enlarged second trial was designed specifically to detect differences between the two strains. The overall incidences of general symptoms of side-effects were not significantly different for subjects receiving either test strain compared to placebo recipients. However, the *aroC ompC ompF* strain was superior from the point of view of inducing an immune response. See the "Discussion and Overall Conclusions" section on page 41 of the report of the second study, where it is stated that:

> "The PTL-ETEC-003 construct was superior to the PTL-ETEC-002 construct in its ability to induce both mucosal and systemic immune responses to CFA/II. PTL-ETEC-003 also exhibited a more sustained intestinal colonization than PTL-ETEC-002."

7. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further these statements are made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

United States Code, and that such wilful false statements may jeopardise the validity of this Declaration, the patent application, or any patents issuing thereon.

Declared this day of May 2003

Michael Darsley, Ph.D.